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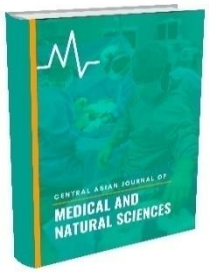

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Study of Some Ginkgo Biloba Contents and Effect of their Extracts as Antioxidants and Antibacterial

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Abstract: Biloba ginkgo leaves are one of the most popular herbal medicines. The purpose of this study was to use ginkgo Biloba leaf extracts to find out how many free amino acids are in a sample. Phenolic compounds, particularly flavonoids, were estimated in the two types of extracts using the high-performance liquid chromatography device (HPLC) and many other important biologically active factors due to their benefits to human health and antioxidant and antibacterial effects. Aqueous extracts and ethanol showed nine types of amino acids, the highest of which was cysteine. In addition to containing good numbers and quantities of phenolic compounds, many of which contain multiple biological properties such as antioxidant and antibacterial, this research has been carried out. An antioxidant analysis showed that ethanol extract showed more antioxidant properties than aqueous extract. However, compared to vitamin C measurement, both extract sections were able to inhibit a good ratio of 1, 1-diphenyl 2-picrylhydrazyl free radical (DPPH) with a good excellent ratio balance. The maximum activity of DPPH root rickets for vitamin C (69.4) ppm, ethanol extract (185.58) ppm, and aqueous extraction (251) ppm were concentrated. The antibacterial activity showed a maximum inhibition area of 24 mm for Staphylococcus aureus as Shigella flexneri at a concentration of 500 ppm.

Key words: Ginkgo biloba, Amino acids, Phenolic compounds, Antioxidants, Antibacterial.

Introduction:

Ginkgo Biloba is a tree species with a rich, and vibrant history of usage for se medicinal purposes because of the various benefits to human health it can provide. The name ginkgo is derived from the Japanese name Yin-Kwo (silver fruit), while the title Biloba refers to the pelvic shape of the leaves. The species exists without interruption for 270 million years without changes and is the oldest tree in the world. As a result, it is classified in a separate section: Ginkgo, with the presence of the existing

species G. Biloba. The hallmark of the gene is that it is coniferouside [1]. Researchers are still studying its mode of action, still discovering new beneficial properties. Active components in Ginkgo biloba leaves extracts, such as terpenoids and flavonoids, possess antioxidant activity [2]. Studies also show the ability of these extracts to enhance blood circulation, reduce the risk of clotting, support capillary walls and their flexibility, and shield neurons from harm, which could be brought about when deprived of oxygen[3]. Protein, lipids, carbs, vitamins, organic acids, polyphenols, beta carotene, flavonoids, and terpenoids are abundant in Ginkgo Biloba leaves [4]. Aside from the well-known flavonol glycosides and terpene lactones, amino acids in Ginkgo Biloba leaves are often found in foods, beverages, and pharmaceuticals [5]. Amino acids (AAs) are chemical molecules with both amino and acid groups[6]. Amino acids are essential life-supporting building blocks. Their function in protein synthesis is widely understood. Still, they also various other intracellular metabolic pathways that support cell processes organismal functions, such as ATP generation, nucleotide synthesis, and redox balancing. Immune cells rely on this pathway amended biomass, as well as to alter their metabolism after activation to sustain their growth, proliferation, and effector activities. Beyond enhanced protein synthesis essential amino acid metabolism is important for this metabolic rewiring and supports various immune cell functions[7][8].

Flavonoids are a diverse group of secondary metabolites found in many plants. It has a flavone structure, which means two benzene rings (A and B rings) are linked by a pyran ring (C ring), which may contain hydroxyl, methoxy, methyl, isoamyl, and other substituents[9]. Flavonoids give plants color flavors and pharmacological properties [10]. Fruits and vegetables are the primary sources of flavonoids [11]. Flavonoids are antioxidants with a lot of power [12], Plants are protected from adverse environmental conditions [13], in addition, flavonoids have biological properties such as improved blood circulation, cholesterol reduction, UV protection, angiogenesis inhibition, antimicrobial, and anti-inflammatory properties[9]. As a result, they have gotten a lot of attention. They have been tested in of many epidemiological and experimental studies to see if they can help with a variety of acute and chronic human disorders [14]. Flavonoids have been proven to have anti-inflammatory and immunomodulatory properties in vitro and animal experiments [15] and potent anticancer properties [14] [16] [17]. Antioxidants are substances that can counteract the oxidative effects of free radicals and other oxidants in living systems [18]. Antioxidants are divided into endogenous and exogenous, based on their presence in the human body[19][20]. They are beneficial to various human products due to their unique chemical and biological features [21]. The search for new and natural antioxidants from dietary plants is gaining traction because they can protect the human body from oxidative damage to biological macromolecules such as lipid, protein, and nucleic acid, which is primarily caused by secondary metabolism [22].

Fig. (1): Ginkgo Biloba plant leaves [23]

Material and Methods:

1- Collection and preparation of Ginkgo Biloba Leave Extract:

The dried leaves of Ginkgo were collected from the north of Baghdad, Iraq, in September 2021, then washed with de-ionized water and dried in the shade for several days at the temperature of the room, and then ground the sample to powder. The conventional Soxhlet extraction method was applied to samples according to [24] for obtaining aqueous and alcohol extracts.

2- Determination of Amino Acids and Phenols concentrations:

Using an HPLC system (Sykam s3210, Germany) with a C18 column (4.6mm × 150 mm, 5µm), all the standard materials used were purely 99% of Samarra Pharmaceuticals. For Amino Acids Mobile phase (acetonitrile: H_2O) (5: 95) respectively, Flow Rate (2 mL/min) ,Detection fluorescence system (340nm-450nm) [25]. For Phenols mobile phase (H_2O: acetonitrile) (70:30) respectively, Flow Rate (1.2 mL/min), Detection fluorescence system (260nm-310nm) [26].

3- Antibacterial Activity studies:

Many microorganisms were used as bacteria, antibacterial activity evaluation of bacteria from selected microorganisms was performed using a spreading tablet, and antimicrobial activity was done to Gingko extracts using a well-hired deployment method [27][28]. The study included testing in-kind water and alcohol extracts from the ginkgo plant in inhibiting the growth of bacteria causing diseases positive for Kram dye outside the living body; depending on the McFarland method, samples were loaded on cellulose tablets in diameter (5mm) bacterial insulation was developed, and 0.5 was transported 1 ml of bacterial and bacterial filaments equivalent to (1.5×10^8) cells/ml to the dish that contains the center of Müller Hanton's dens, diffuse, leaving the dish for 15-20 minutes at room temperature until the implant is absorbed Bacterial, tablets loaded with extract and plates are incubated at 37^OC temperature. For 24 hours, the diameters of the tablet areas are measured around the chips for each type of bacteria used.

4- Determination of free radical scavenging activity:

Ginkgo Biloba extracts' free radical scavenging activity was determined using the DPPH. At varied concentrations (12.5, 25, 50, 100, and 200 g/mL), the extract was evaluated. To 3 mL of daily prepared methanol and DPPH solution, a (150L) methanol solution containing various volumes of sample solution was added. At 517 nm, 20 minutes after the first mixing, optical density changes were recorded, with a UV spectrophotometer; Vitamin C been used as a benchmark. The sample's scavenging activity was determined by comparing its absorbance to that of the DPPH reference solution The following formula was used to calculate the radical scavenging activity percentage of the DPPH: **% Antiradical activity = (A_o – A_C) /A_o× 100**

Where Ao is the absorbance of the control and Ac is the absorbance of the sample or standard in the presence of the control. The results of three independent trials were averaged and expressed as a percentage of mean radical scavenging activity [29].

3. Results and Discussions:

The results revealed that G. Biloba leaf extracts were high in free amino acids, especially those considered essential, such as) Arginine, Valine, Isoleucine, Leucine, Phenylalanine, Tyrosine), addition to (Cysteine, Alanine, Glutamic acid). The total amount of amino acids in the sample ranged from (250.197 to 1905.307) ppm in aqueous extract, while it was from (270.346 to 1277.005) ppm in the alcoholic extract, according to the table (1) and fig. (2).

Table (1) Concentration of amino acids in the aqueous and alcohol extracts of the Ginkgo biloba.

Amino acid	Aqueous extract			Alcoholic extract			
	Retain. Time [min]	Amount [ppm]	Amount [%]	Retain. Time [min]	Amount [ppm]	Amount [%]	Mean ± Sd
Alanine	-	-	-	9.868	427.075	10.4	10.4 ± 7.4
Arginine	9.132	250.197	5.1	8.976	709.506	17.3	11.2 ± 8.7
Cysteine	11.280	1905.307	39.0	11.212	1277.005	31.2	35.1 ± 5.5
Glutamic acid	-	-	-	7.804	1023.273	25.0	25 ± 17.7
Isoleucine	13.044	274.827	5.6	-	-	-	5.6 ± 4.0
Leucine	13.256	321.532	6.6	-	-	-	6.6 ± 4.0
Phenylalanine	14.024	809.927	16.6	13.520	270.346	6.6	11.6 ± 7.1
Tyrosine	12.464	295.353	6.0	12.228	382.716	9.4	7.7 ± 2.4
Valine	11.956	1028.445	21.1	-	-	-	21.1 ± 15.0

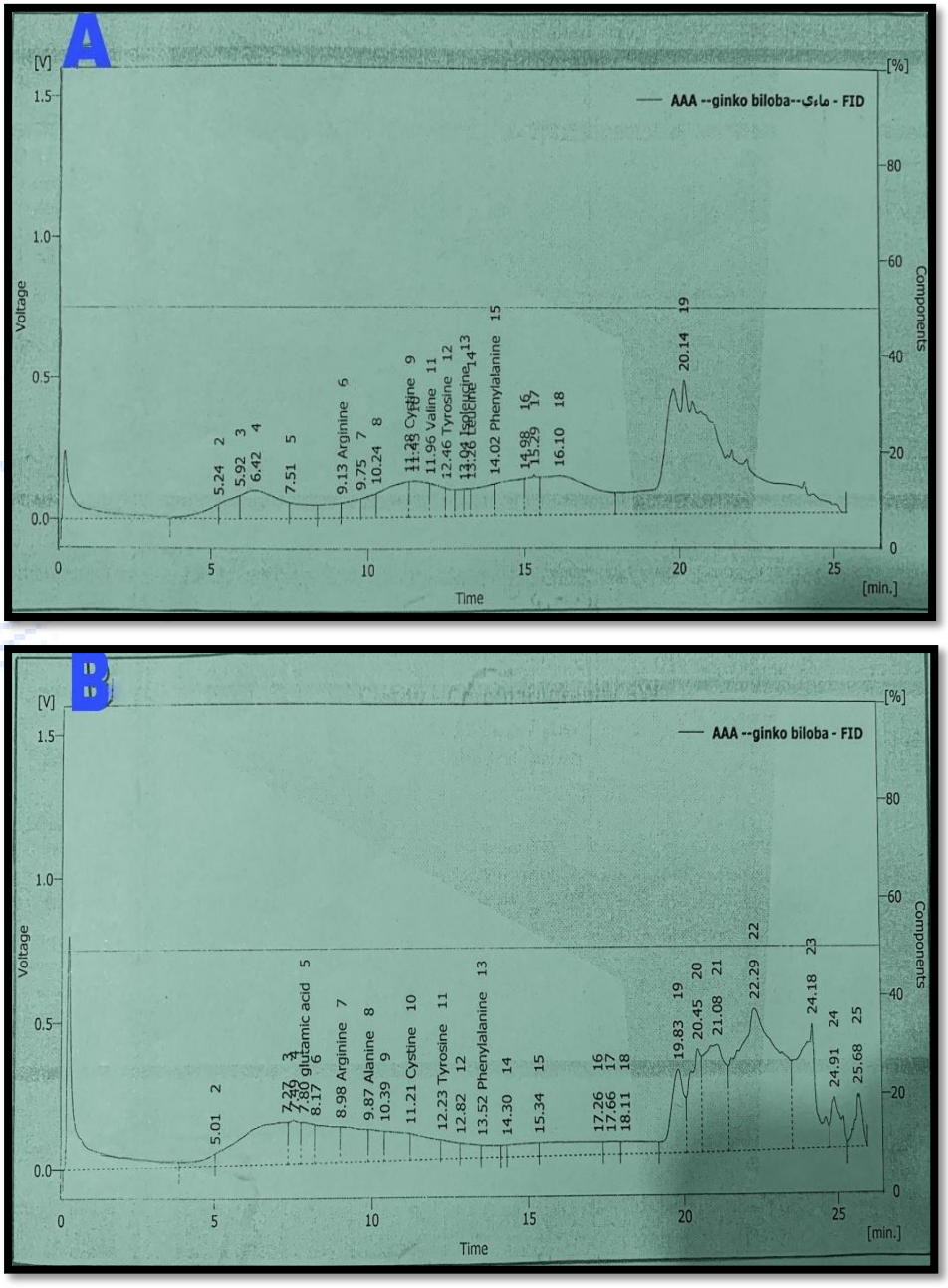


Fig. (2) HPLC analysis for (A) Amino acid in the aqueous extract. (B) Amino acid in ethanol extract.

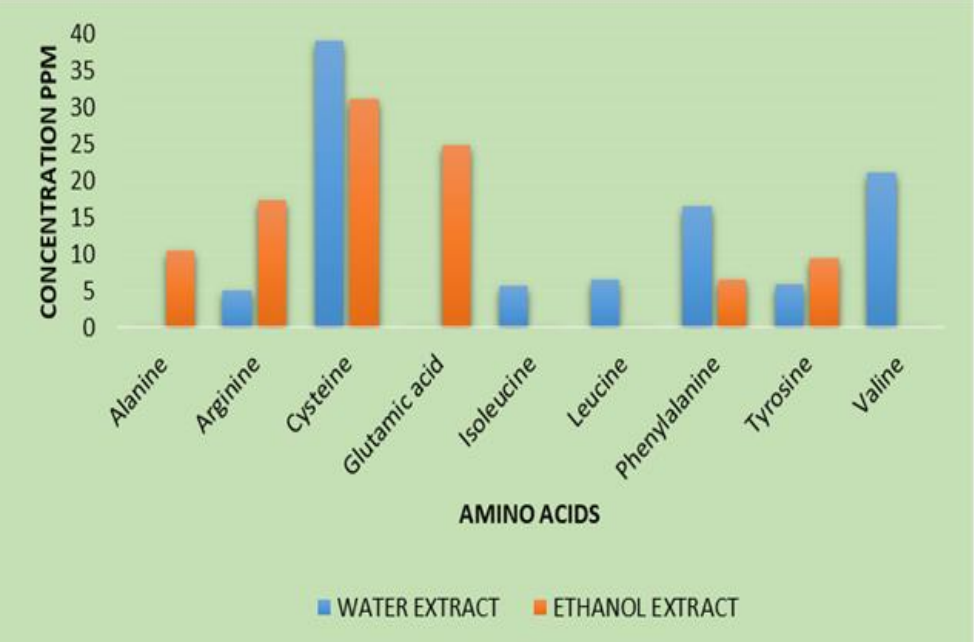


Fig. (3) Graph shows the concentrations of amino acids in extractors

Amino acids are essential building blocks as a source of energy for cell survival, regeneration, and growth. Each amino acid has a distinct carbon structure, an amino group, and a carboxylic acid. Humans use 20 amino acids; most amino of these may be made naturally, but nine are "essential," meaning they must be consumed. Amino acids are important energy sources being protein-building components (ketogenic, glycogenic, or both), are recycled as needed, and serve as building blocks for Krebs's cycle intermediates and other metabolites [30]. Amino acids (AA) are used of product protein and a variety of low-molecular-weight molecules with significant physiological significance. Nutritionally, amino acids are necessary for maximal growth and lactation and optimal health, well-being, and reproduction [31]. Certain amino acids can be transformed into other amino acids, proteins, glucose, fatty acids, or ketones in the human body [32]. Chemical messengers in the neurological system (neurotransmitters) and precursors to nucleic acids, which are components of DNA: glutamate [33]. Alanine has a direct role in gluconeogenesis; transamination [34]. Arginine has properties of antioxidant; regulation of hormone secretion; ammonia detoxification; regulation of gene expression and immune function [35]. Cysteine is a disulfide bond in proteins that allows sulfur to be transported [36].

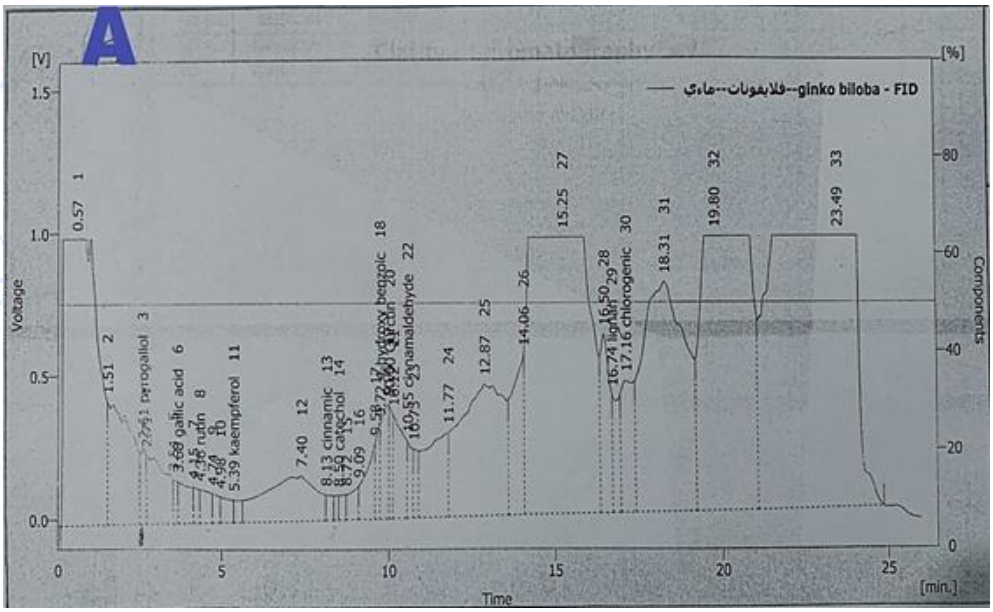
Glutamine: The production of arginine connects the urea and Krebs cycles. Isoleucine Synthesis of glutamine and alanine. Leucine; Regulation of protein turnover and gene expression; activator of glutamate. Phenylalanine; synthesis of tyrosine; neurological development [37]. Tyrosine is a precursor of dopamine, epinephrine, norepinephrine, and thyroxin [38]. The HPLC analysis of Ginkgo Biloba Leave Extracts investigated important antioxidant phenolic compounds in this study. We used several different standard solutions for phenols. The results showed that G. Biloba leaves interesting focus extracts because it contains a large number of phenol, which per compound was calculated by comparing the area of the pick of the standard substance with the size of the picked-choice for the desired combination and according to the following equation: **C (sample) = [A (sample) ×A (standard)] /C (average)**

In the aqueous extract, the concentration of (4-hydroxy benzoic) was the highest and had the best separation of the peak at retention time (9.720 min) according to the peak of standard substances, and the presence of all phenolic compounds at range retention time (2-18min), Figure (4) and table (2). In

the alcohol extract, the concentration of (4-hydroxy benzoic) too was the highest and had the best separation of the peak at retention time (10.020 min) according to the rise of standard substances, and the presence of all phenolic compounds at the range retention time (2-18min), Figure (4) and table (2).

Table (2) Concentration of phenolic compounds in the aqueous and alcohol extracts of the Ginkgo biloba.

phenolic compounds	Result Aqueous Extract			Result Alcohol Extract			Retention Time [Min] Standard
	Area[mV.s]	conc. [µg.mL^{-1}]	Retention Time [Min]	Area[mV.s]	conc.[µg.mL^{-1}]	Retention Time [Min]	
Pyrogallol	3131.303	0.183	2.608	926.427	0.054	2.408	2.782
Gallic acid	3737.326	0.205	3.684	352.120	0.029	3.876	3.607
Rutin	2564.507	***	4.356	1159.385	***	4.408	***
Kaempferol	1234.370	0.229	5.388	1995.025	0.370	5.444	5.448
Cinnamic	1269.683	6.749	8.132	964.322	5.126	8.124	8.0
Catechol	716.155	13.911	8.500	859.589	16.697	8.496	8.300
4-Hydroxy benzoic	2555.536	293.470	9.720	2667.033	306.274	10.020	9.953
Quercitin	2702.706	10.106	10.000	1357.784	5.077	10.144	10.092
Cinnamaldehyde	2735.988	40.639	10.548	3248.364	48.250	10.488	10.403
Lignin	5735.893	2.722	16.744	3036.491	1.441	16.852	16.740
Chlorogenic	11810.94	261.338	17.160	3123.383	69.110	16.852	17.083
Nigellone	-	-	-	6805.011	12.282	17.036	17.280
Eugenol	-	-	-	2405.560	2.985	13.804	13.890



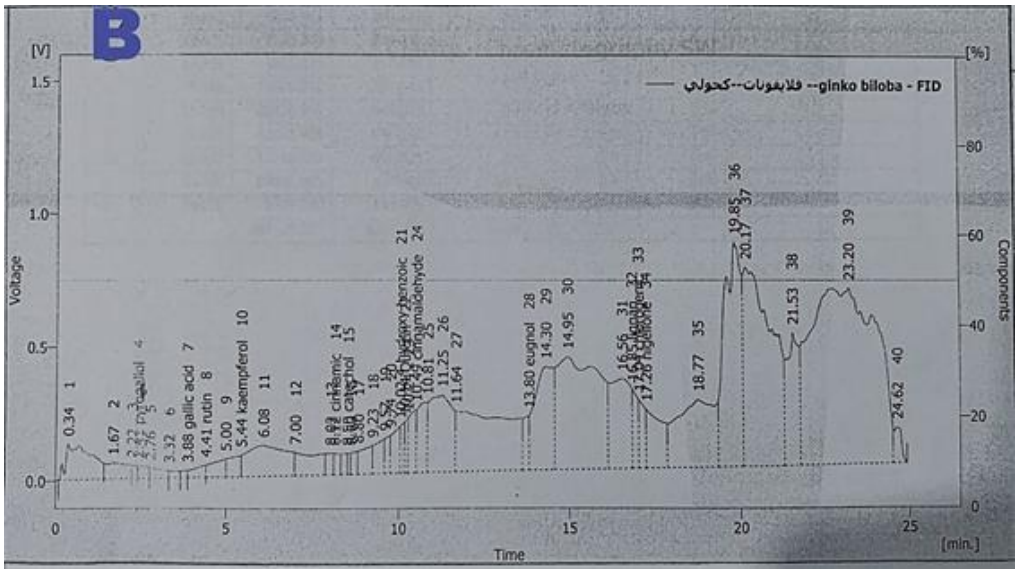


Fig. (4) HPLC analysis for (A) Phenolic compounds in the aqueous extract. (B) Phenolic compounds in ethanol extract.

For the research and development of Ginkgo biloba L., it is critical to get a systematic understanding of flavonoids and their actions. Many studies and research have shown that flavonoids have important vital role in treating many diseases. An association has been observed between the chemical composition of flavonoids and their therapeutic properties [39], as an increase in hydroxyl totals increases anti-tumor activit rise he increase in the number of mitochondrial accommodations increases anti-cancer someivity [40], Some flavonoids also have anti-inflammatory effects [41], for allergies [42], for microbes [43], and viruses [44]. Preliminary screening was performed against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella sp., and Candida albicans* was performed. The initial examination was performed against *Orius staphylococcus*, *staphylococcus epidermis*, *E. coli*, *Klebsiella sp.*, and *Candida Albicans*. The initial concentrations taken from the extractors were (500,250, 125, 6 2.5 ppm. The results indicated that there was no effect of aqueous extract for any type of previous bacteria and that the results of the alcohol extract were as shown in the table. The efficacy of the aqueous extract was subsequently examined at a concentration of 1500 ppm and its effect on inhibiting *staphylococcus aureus* was only at a magnitude of 14 mm table(3).

Table (3) Values the standard deviation and mean of the aqueous and alcohol extracts of the Ginkgo biloba.

Sample No.				S1	S2	S3	S4
Aqueous Extract	Gram-positive	*S.aureus*	Mean	-	-	-	-
			Sd	-	-	-	-
		S.epidermidis	Men	-	-	-	-
			Sd	-	-	-	-
	Gram-Negative	*E .coli*	Mean	-	-	-	-
			Sd	-	-	-	-
		Klebsiella sp.	Mean	-	-	-	-
			Sd	-	-	-	-
	Fungi	*C.albicans*	Mean	-	-	-	-
			Sd	-	-	-	-
	Gram-positive	*S.aureus*	Mean	24	12	10	-
			Sd	2.11	0.92	1.24	-

Alcohol Extract		*S.epidermidis*	Mean	12	10	-	-
			Sd	1.43	1.33	-	-
	Gram-Negative	*E .coli*	Mean	17	15	14	-
			Sd	1.56	1.89	1.22	-
		Klebsiella sp.	Mean	-	-	-	-
			Sd	-	-	-	-
	Fungi	*C.albicans*	Mean	14	11	10	-
			Sd	1.16	0.96	1.11	-

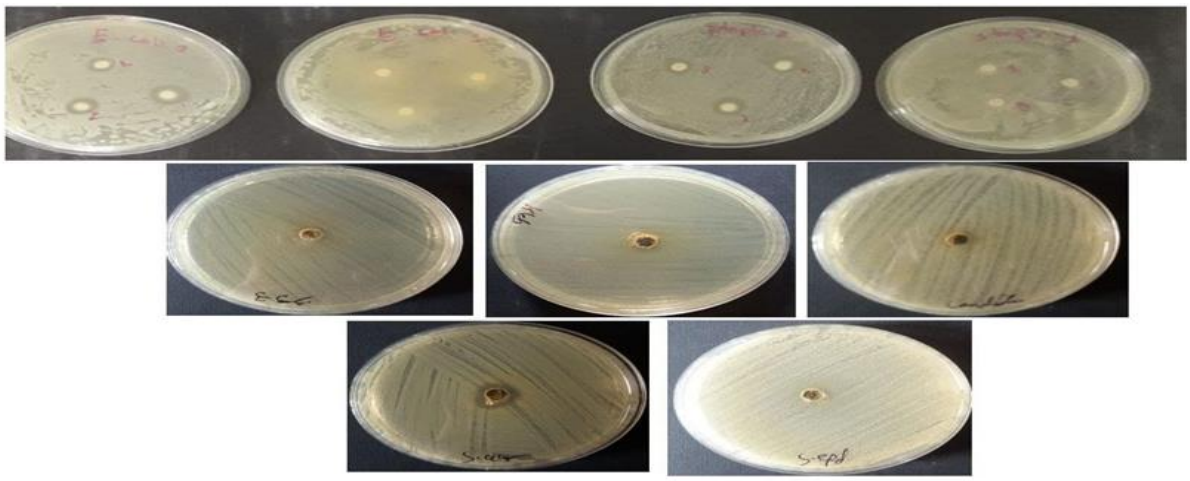


Fig. (5) Inhibition zones caused by Ginkgo Biloba extracts Against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella sp.,* and *Candida Albicans* after 24hrs of the incubation period.

There are different inhibition mechanisms against microorganisms suchirected as cell membrane damage, inhibition of protein synthesis, and disruption of cell biological functions and cell membranes by specific enzymes. The nature of bacterial cell wall is influenced by the presence of compound like α-Pinene and β-pinene (pinene-type monoterpene hydrocarbons) which is involved in the membrane cell wall disruption by the lipophilic compounds [45]. In addition, the nature of gram-negative bacteria cell walls with high lipid content (up to 20 %) compared with 0-2% for gram-positive is responsible for the resistance of microorganisms such as Escherichia coli, In contrast, positive gram-positive bacteria show more sensitivity to the antimicrobial compounds found in extracts [40]. It is concluded that the alcohol extract of the Ginko has antimicrobial activity against gram-positive bacteria higher than gram-negative bacteria that resist both water extracts and ethanol [46].

Free radical scavenging activity: The DPPH radical scavenging assay is a sensitive antioxidant assay substrate unaffected by the substrate's polarity. The stable free radical DPPH can take an electron or a hydrogen radical to form a stable diamagnetic molecule. The high phenolic content of the extracts may explain they are great antioxidant action. Based on these findings, the extracts and the mixed sample both showed antioxidant activity against DPPH radicals, composite action activity. Therefore, this exploratory research reveals the intriguing anti-oxidant stress function of Gingko leaves extracts, suggesting their promising uses as a therapeutic source for the treatment and prevention. It offers uses benefits therapeutic medicinal illnesses caused by free radicals From Fig(6), data showed a good percentage of antioxidants that the two extracts contain compared with vitamin C, a strong and effective antioxidant [47].

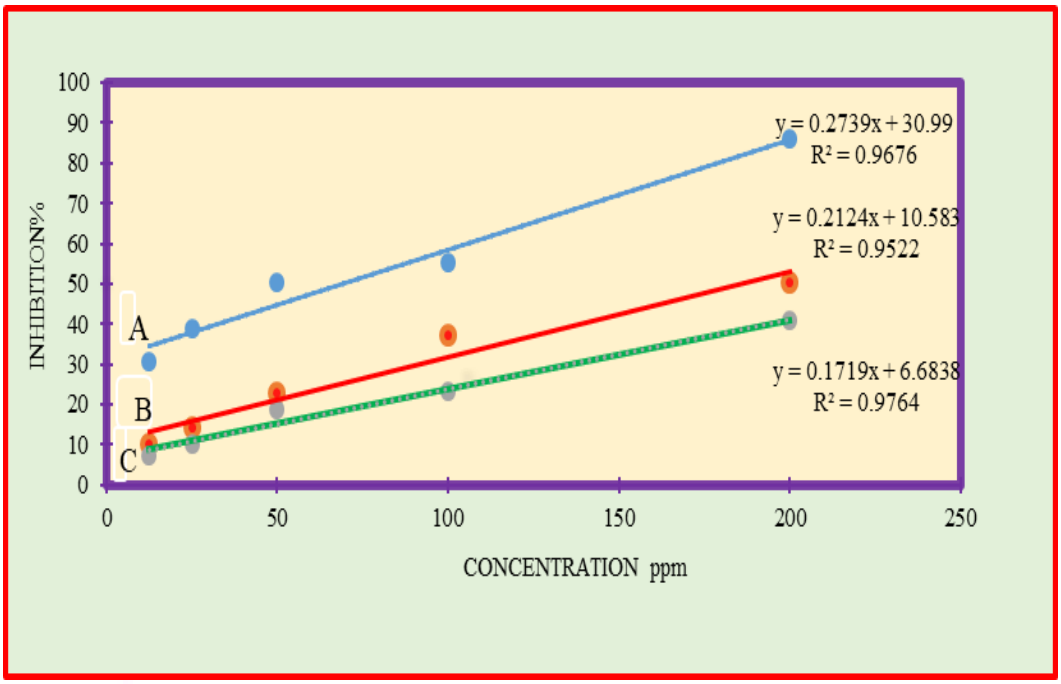


Fig. (6): DPPH Free radical scavenging activities of Ginkgo Biloba extracts compared with Vitamin C. A: Vitamin C (IC50=69.4 ppm), B: Ethanol Extract (IC_{50}=185.58 ppm), C: Water Extract (IC50=251 ppm).

The results showed the presence of DPPH radical scavenging activity for water extracts and ethanol from Biloba ginkgo leaves. The highest% scavenging activity was observed in the ethanol extract according to their different concentration of 10µg/ml to 60 µg/ml. The high DPPH scavenging activity of Ginkgo biloba leaf extracts aligns with the reports [48]. The extracts were able to donate a hydrogen atom to DPPH and change the colour. The increasing intensity of the color is directly proportional to the inhibition of DPPH [49].

Conclusion:

As a result of this research, it was confirmed that Ginkgo Biloba leaf extracts contain abundant amounts of amino acids, flavonoids, and phenols. This high content of flavonoids and phenols is the primary driver of their antioxidant activities. Ethanol also showed 99% more extraction of phenolic content than aqueous extraction, so ethanol extract showed the best antioxidant properties. The increasing concentration of the extract is increasingly preventing DPPH activity. Ethanol extracts alsohad a clearance an apparent effect on bacterial inhibition compared to aqueous extracts.

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